December 1914. | Chemical Series. | Vol. IV, No. 1

Memoirs of the Department of Agriculture in India

THE GASES OF SWAMP RICE SOILS

PART II

THEIR UTILIZATION FOR THE AERATION OF THE ROOTS OF THE CROP

BY

W. H. HARRISON, M.Sc.

Government Agricultural Chemist, Madras

AND

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AGRICULTURAL RESEARCH INSTITUTE, PUSA

PRINTED AND PUBLISHED FOR

THE IMPERIAL DEPARTMENT OF AGRICULTURE IN INDIA

BY

THACKER, SPINK & CO., CALCUTTA

W. THACKER & CO., 2, CREED LANE, LONDON

Price, Re. 1 or 1s. 6d.

MEMOIRS OF THE
DEPARTMENT OF AGRICULTURE
IN INDIA

CHEMICAL SERIES

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EDITED BY

The Council of the Pusa Agricultural Research Institute.

CONTENTS.

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Introductory.

In a recently published paper[1] dealing with the gases of Swamp Rice Soils, we showed that the gases formed in the soil appeared to have an important connection with the aeration of the roots of the crop.

The chief facts which led us to this conclusion may be briefly stated as follows :—

1. The gases which occur in the soil consist mainly of methane and nitrogen, together with small amounts of carbon-dioxide and hydrogen, and these, with the exception of nitrogen, may be looked upon as the characteristic soil gases.[2]

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[1] Harrison and Subramania. "The Gases of Swamp Rice Soils," *Mem. Dept. Agri. India, Chem. Ser.*, Vol. III, No. 3.

[2] *Ibid*, p. 68.

2. The surface of the soil immediately in contact with the irrigation water is covered by an organized film which evolves considerable quantities of gas, of which oxygen is one of the chief constituents and which is utilized for the aeration of the roots of the rice crop. Further a careful examination of this gas thus evolved shows that it contains no trace of the characteristic soil gases, so long as the surface of the soil is not disturbed, and it must therefore be concluded, that either the production of methane, etc., in the soil is not sufficiently large to cause it to be discharged through the surface of the soil, or that the organized film possesses the power of arresting and assimilating these gases.[1]

3. That the first supposition is incorrect is demonstrated by the fact that when the growth of the film is prevented, *e.g.*, by the use of copper sulphate in the irrigation water, the characteristic soil gases escape from the soil in abundance and can easily be collected.[2]

4. That the second hypothesis is probably correct is shown by the facts (*a*) that the addition of green manure to the soil greatly increases the production of methane ;[3] and (*b*) that this increased production of gas coincides with an increased evolution of oxygen from the surface film.[4]

Consequently it appeared very probable that the organisms forming the surface film possess the power of utilizing the characteristic soil gases so as to lead to an increased output of oxygen at the surface of the soil. This result might possibly be brought about by one, or both of two methods. Either the gases may be utilized directly in the metabolism of the organisms or they may be oxidized at the expense of the oxygen dissolved in the irrigation water, and

---

1 Harrison and Subramania. " The Gases of Swamp Rice Soils," *Mem. Dep. Agri. India, Chem. Ser.*, Vol. III, No. 3, pp. 89—93.

2 *Ibid*, p. 77.

3 *Ibid*, p. 79.

4 *Ibid*, pp. 104-6.

the carbon-dioxide thus produced be in turn decomposed by the green algæ present in the film with the liberation of gaseous oxygen.

The first supposition does not appear to be very probable, because then the sole source of the evolved oxygen could only be the carbon-dioxide dissolved in the irrigation water and the action of green manures in increasing the output of oxygen would appear to be very obscure. On the other hand, the work of Söhngen,[1] Kaserer,[2] and others, on the bacterial oxidation of methane and hydrogen makes the second supposition appear very probable and would account at the same time for the effect of green-manuring.

In order to test these hypotheses a series of experiments were initiated to determine the action of the film on the soil gases and the nature of the agents to which the changes produced could be ascribed. The results of this investigation are detailed in the following pages.

Experimental.

The Action of the Surface Film on the Soil Gases.

Several different types of the surface film from Swamp Rice Soils were sealed up in glass vessels with a few cc. of a sterile mineral nutrient solution* and a known volume of a mixture of the soil gases. The usual method adopted in carrying out these experiments with the film was to take a cylindrical separating funnel A, the stopper of which is replaced by a rubber cork through which passed a capillary glass tap B and to which was sealed a small bulb C filled with cotton-wool. The cylinder was completely filled with mineral nutrient solution by suction at C and the tap B closed. The whole was then sterilized in the autoclave, the tap D

[1] Söhngen, *Centbl. Bakt.* 2 abt., 15 (1905), p. 573.

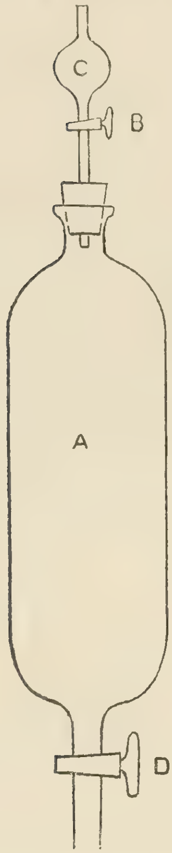
[2] Kaserer, *Centbl. Bakt.* 2 abt, 15 (1905), p. 573, 16 (1906), p. 681.

* *Note.*—In all these experiments Kaserer's (*loc. cit*) mineral solution was used consisting of—

K_2HPO_4	0·05%
$MgSO_4$	0·02%
NH_4Cl	0·10%
$NaHCO_3$	0·05%
$FeCl_3$	trace.

Other solutions were tried, but the above was found most satisfactory.

being allowed to remain open and to dip in a beaker of the solution.



When cool, any gas remaining in A was removed by gentle suction through C and the tap D was closed. The film was introduced into the body of the apparatus by momentarily removing the cork, and the gas was allowed to pass in through C, the flow being regulated by the tap D. There was no difficulty met with in preventing the film blocking up the tap D during the filling if the apparatus was maintained in a sloping position. These vessels were kept submerged under water and exposed to sunlight for about 8 days. At the end of this period the tap D was opened, whilst still under the water, and after equalising the internal and external pressure, the volume of the residual gas was measured and an analysis made. The observed decrease in volume varied considerably, but in every case the analysis disclosed considerable changes in the composition of the original gas.

The results of two typical experiments are given in Table I.

TABLE I.

Showing the effect of the film on the soil gases in sunlight and in the presence of oxygen.

cc. NT. & P.

	Exp. No. 1.			Exp. No. 2.		
	Before.	After.	Diff.	Before.	After.	Diff.
CO_2	210·6	60·3	−150·3	49·5	*Nil.*	−49·5
O	156·6	285·8	+129·2	29·1	40·5	+11·4
CH_4	98·8	66·4	− 32·4	56·1	26·9	−29·2
H	*Nil.*	..	..	4·0	..	−4·0
N	24·0	27·7	+ 3·7	3·4	4·6	+1·2
TOTAL ..	490·0	440·2	−49·8	142·1	72·0	−70·1

In every case the volume of oxygen increased, whereas that of methane and carbon-dioxide decreased. Considering experiment No. 1, if the methane which disappeared were oxidized to carbon-dioxide and water, 64·8 cc. of oxygen would be required and there would be 32·4 cc. of carbon-dioxide formed. Consequently, during the whole course of the experiment there would be present 243 cc. of CO_2, but of this amount only 182·7 cc. have disappeared. On the assumption that the CO_2 which disappears is decomposed by chlorophyllaceous algæ it would yield 182·7 cc. of oxygen, thus making the total volume of oxygen present during the course of the experiment equal to 339·3 cc. From this volume it is necessary to deduct the 64·8 cc. utilized for the oxidation of the CH_4, leaving a balance of 274·5 cc. at the end of the experiment. As a matter of fact 285·8 cc. were actually found and the difference is probably accounted for by the CO_2 dissolved in the small amount of liquid present in the tube and which would not appear in the analysis.

Applying the same argument to Expt. II, the volume of oxygen which was theoretically recoverable was 47 cc. and 40·5 cc. were actually recovered.

Thus it is possible, on a quantitative basis, to explain the changes which occur by assuming that the film is capable of oxidizing the methane of the soil gases on the one hand, and on the other of decomposing the CO_2 thus produced with the liberation of an equal volume of oxygen. Green algæ are generally a constituent of the film, whilst diatoms are invariably present, and it would appear probable that the decomposition of the carbon-dioxide was due to these organisms. Further the film always contains bacterial growths and the oxidation of the methane and hydrogen is probably brought about by their agency.

If this were the case then the decomposition of the CO_2 should be inhibited by carrying out the experiments in the dark and the action of the film merely confined to oxidizing the methane. To test this the experiments were repeated in an identical manner but

the glass vessels were kept in darkness in the incubator at 32°C. for a period of about 10 days. At the end of that time the volume of gas remaining was measured and analysed with the following results :—

TABLE II.

Showing the action of the film on CO_2 and CH_4 in the presence of O and in darkness.

cc. NT. & P.

EXPERIMENT.	FILM No. I.			FILM No. II.			FILM No. III.			FILM No. IV.		
Type of film used.	A whitish growth containing very little green algæ.			Consisting mainly of nostoc.			Mainly oscillatoria and a white growth.			A brownish growth containing a large number of diatoms and a white growth.		
	Before.	After.	Diff.	Before.	After.	Diff.	Before.	After.	Diff.	Before.	After.	Diff.
CO_2 ..	26·7	39·4	+12·7	26·7	26·8	+ 0·1	26·7	38·0	+11·3	18·0	21·4	+ 3·4
O ..	77·8	41·1	−36·7	77·8	24·2	−53·6	77·8	31·5	−46·3	60·1	45·3	−14·8
CH_4 ..	28·9	5·3	−23·6	28·9	0·6	−28·3	28·9	0·9	−28·0	34·5	26·9	− 7·6
N ..	6·6	6·4	− 0·2	6·6	8·2	+ 1·6	6·6	6·2	− 0·4	7·4	6·4	− 1·0
TOTAL	140·0	92·2	−47·8	140·0	59·8	−80·2	140·0	76·6	−63·4	120·0	100·0	−20·0

These results clearly show that the oxidation of the methane was not affected by the absence of light, whilst in no case was there a diminution in the original volume of carbon-dioxide present, but, on the contrary, usually a very decided increase. The conclusion is evident, that the action of the film, so far as methane is concerned, is mainly due to two separate and distinct agencies. One possessing the power of oxidizing methane to carbon-dioxide, and which is independent of the action of light. The other being capable of decomposing carbon-dioxide with the evolution of oxygen and dependent upon the presence of sunlight. The agents in the latter case are undoubtedly the green algæ, diatoms, etc., which are universally present in the films.

In this series of experiments the production of carbon-dioxide is less than the amount which would be expected from the volume of methane which disappears, and this fact would argue the presence of a third agent in the film which possesses the power of assimilating carbon-dioxide as such without a corresponding liberation of oxygen.

The photo-synthetic action of the green algæ of the film is easily demonstrated by confining the latter in vessels filled with a gaseous mixture of carbon-dioxide and nitrogen and exposing them to sunlight for several days. The result of a typical experiment is given in the following table :—

TABLE III.

Showing the action of the film on CO_2 in sunlight.

cc. NT. & P.

	Before.	After.	Diff.
CO_2	87·4	6·8	−80·6
O	9·6	81·2	+71·6
N	53·0	52·0	−1·0

Further, it was noticed that hydrogen also disappeared whenever it was present in the mixed gases, and in such proportion to the oxygen that it was apparent that oxidation to water had occurred. As an example of this action the result of a typical experiment is given below :—

TABLE IV.

Showing the oxidizing action of the film on hydrogen in the dark.

cc. NT. & P.

	Before.	After.	Diff.
CO_2	1·9	4·3	+2·4
O	190·8	130·0	−60·8
CH_4	26·8	27·1	+0·3
H	136·2	..	−136·2
TOTAL ..	355·7	161·4	−194·3

The ratio of the number of cc. of oxygen which have disappeared to those of hydrogen is O/H = 1/2·2, a proportion which points to the process being one of oxidation only.

The experiments detailed above made it quite clear that the film (*a*) exerts a strong action on the soil gases, oxidizing the methane and hydrogen to carbon-dioxide and water, (*b*) can carry on the ordinary photo-synthetical processes of assimilating carbon-dioxide and liberating oxygen owing to the presence in it of chlorophyllaceous organisms, and (*c*) probably contains organisms capable of assimilating carbon-dioxide as such.

As the actions (*a*) and (*c*) are not inhibited by absence of light it was probable that the organisms bringing these changes about were bacterial in character and this hypothesis determined the trend of the latter portion of this investigation, the results of which are detailed in the following pages.

The oxidation of methane.

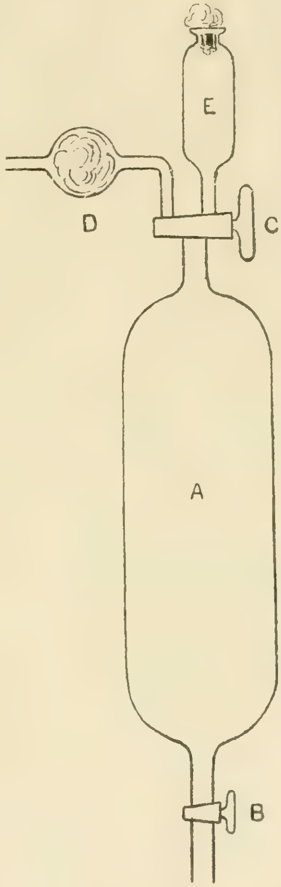
About 25 cc. of Kaserer's mineral nutrient solution was introduced into a Botkin culture flask, the gas leading tubes of which were plugged with sterile cotton-wool, and the whole was sterilized in the autoclave.

The solution was then inoculated with a very small piece of a film and a mixture of oxygen, nitrogen and methane was slowly bubbled through until the air in the flasks was displaced. After incubation at 32° C. for several days, a strong white film had formed inside the flasks and there was a partial vacuum caused by the oxidation of the methane—

$$\begin{array}{ccccccc} CH_4 & + & 2O_2 & = & CO_2 & + & 2H_2O \\ 2 \text{ vols.} & + & 4 \text{ vols.} & = & 2 \text{ vols.} & & \end{array}$$

By repeated transfers into fresh solution the crude culture was purified until there appeared to be only very few bacterial species present and the mixed culture thus obtained exerted a strong oxidizing action on methane.

The experimental methods adopted in order to obtain a quantitative measurement of this oxidation were as follows :—



The apparatus used is shown in the annexed sketch and consists of a cylindrical vessel A having a single bored stopcock B sealed on to the lower end, and a double bored one C to the other end. The double bored stopcock communicates on the one hand with the bulb tube D, the bulb of which was filled with cotton-wool, and on the other hand, with a small cylindrical vessel E having a capacity of about 10 ccs. and closed with a plug of sterile cotton-wool. In practice, the vessel A is filled with the mineral nutrient solution by means of suction through D, the tap B being opened and placed in a beaker of the liquid. Tap C is then closed and after introducing a little of the solution into E, the whole is sterilized in the autoclave, the end B remaining in the beaker with the tap open. When cool, a gentle suction at D removes any air remaining in A and then both the taps are closed.

The culture solution in E is inoculated with the crude culture and D is connected to a gas-holder containing the mixed gases. The latter are drawn into the vessel A by opening the taps C and B, the velocity being regulated by tap B. When nearly filled with the gas, the tap B is closed and the vessel A connected to the smaller vessel E. A little gas is expelled from A by the warmth of the hand and on cooling, the contents of the vessel E are drawn into A and the inoculation is accomplished. The taps are then securely closed and the whole apparatus is immersed in a vessel of water and incubated at 32° C. for several days.

At the end of the incubation period the vessel is removed, dried and weighed, and then the tap B is opened under water. Water rushes in to replace the gases which have disappeared and after equalizing the internal and external pressures, the vessel is again weighed. From the increase in weight obtained, the volume of gas which has disappeared is calculated. The volume of residual gas is measured by transference to a gas burette and an analysis is made. The results of several typical experiments with different crude cultures are given below :—

TABLE V.

Showing the oxidation of methane by the crude culture.

cc. NT. & P.

	Exp. I (14 days).			Exp. II (14 days).			Exp. III (21 days).		
	Before.	After.	Diff.	Before.	After.	Diff.	Before.	After.	Diff.
CO_2 ..	1·7	14·7	+13·0	0·4	18·3	+17·9	0·6	14·4	+13·8
O ..	80·8	59·6	−21·2	53·7	14·8	−38·9	80·6	50·5	−30·1
CH_4 ..	51·0	22·1	−28·9	33·2	10·6	−22·6	49·7	32·6	−17·1
N ..	9·3	7·7	−1·6	6·2	3·4	− 2·8	9·2	4·5	− 4·7
TOTAL .	142·8	104·1	− 38·7	93·5	47·1	−46·4	140·1	102·0	−38·1

These experiments show that the bacteria composing the mixed culture exert a very strong oxidizing action on methane, but at the same time it is noticeable that the ratio of the volumes of the methane and oxygen which disappear is not in accordance with the theoretical, being 1/0·7, 1/1·3 and 1/1·8 respectively instead of 1/2. Further in the oxidation of methane the ratio between the CO_2 produced and the oxygen used is 1/2, and in these experiments the ratios found are 1/1·6, 1/2·2 and 1/2·2 respectively, a result much nearer the theoretical than that obtained for the oxygen-methane ratio. These considerations lead to the conclusion that a considerable proportion of methane has been assimilated direct. Söhngen[1]

[1] Söhngen, *loc. cit.*

reports a similar result. In one of his experiments 225 cc. of methane and 148 cc. of oxygen disappeared, whereas only 99 cc. of carbon-dioxide were obtained, and at the same time considerable amounts of organic matter were produced. He concludes that this is due to the direct assimilation of methane.

Although this similarity between our results and those of Söhngen is apparent, yet by following out Söhngen's methods we have been unable to isolate the *Bacterium Methanecus* which he obtained, and neither have we been able by other methods to isolate any bacterium which in pure culture in mineral solution possessed the power of oxidizing methane.

It would therefore appear that there are in these rice soil films a number of species of bacteria acting together in symbiosis, the resultant being the oxidation of methane to CO_2 and the probable formation of some organic matter.

Notwithstanding our failure to isolate a specific methane-oxidizing bacterium, our main contention that this oxidation is due to bacteria must be taken as proved.

A photograph of the organisms present in the crude culture $\times$ 1,000 diameters is given in the plate.

The oxidation of hydrogen.

We have previously referred to the fact that the surface film on rice soils possesses, to a considerable degree, the power to oxidize hydrogen, and, consequently, following out the line of experiment detailed under the section dealing with the oxidation of methane, we obtained crude cultures of bacteria in mineral solution which also possessed the same power in the presence of CO_2 and CH_4. These crude cultures were purified by repeated transfers into sterile mineral nutrient solution contained in Botkin flasks and incubating in an atmosphere of oxygen, hydrogen and methane.

To illustrate the action of the mixed cultures thus obtained the results of two typical experiments are given below ; the method of experiment being that previously described on page 9.

Table VI.

Showing oxidation of hydrogen by crude culture.

cc. NT & P.

	Exp. I (7 days incubation).			Exp. II (14 days incubation).		
	Before.	After.	Diff.	Before.	After.	Diff.
CO_2	1·5	0·8	−0·7	1·3	0·6	−0·7
CH_4	12·6	10·4	−2·2	11·4	6·9	−4·5
O	37·3	31·5	−5·8	33·6	14·4	−19·2
H	35·3	23·0	−12·3	31·8	*Nil.*	−31·8
N	19·3	19·3	*Nil.*	17·4	21·2	+ 3·8
Total ..	106·0	85·0	−21·0	95·5	43·1	−52·4

The ratio of the volumes of oxygen and hydrogen which disappear are approximately 1/2·1 and 1/1·7 respectively as against the 1/2 required theoretically, a result which points to the action being one of oxidation only, particularly as there is no increase in the volume of CO_2 present, as would occur, if the methane were also subject to oxidation.

Further if the assumption is made that the methane is oxidized to CO_2 and the latter assimilated as such, then ratios of the balance of the oxygen used up to the hydrogen which disappears would be approximately 1/9·8 and 1/3·1 respectively. It therefore appeared probable that the mixed culture derived its carbon mainly from the methane and that the oxidation of the hydrogen yielded energy for the metabolic processes.

A photograph of the organisms present in the crude culture × 1,000 diameters is given in the plate.

As our main purpose in this paper is merely to demonstrate the functions of the bacteria present in the films, we do not propose entering into great detail here relating to the functions of the specific bacteria isolated. These we intend publishing at a very early date as a separate paper.

PLATE.

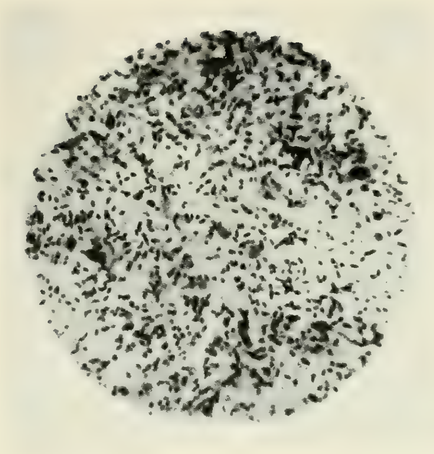


Fig. 1.
Bacteria composing the Crude Culture which oxidizes CH_4 X 1000.

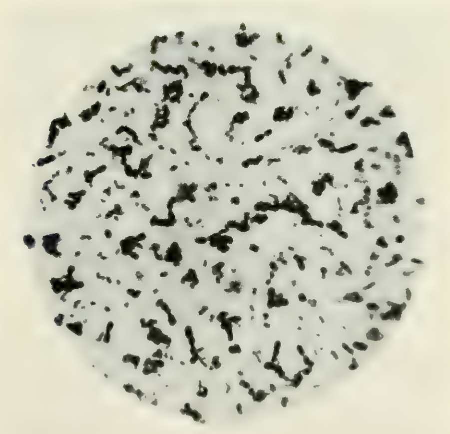


Fig. 2.
Bacteria composing Crude Hydrogen oxidizing Culture X 1000.

Briefly, we have succeeded in isolating an organism which oxidizes hydrogen to water in the presence of small amounts of nitrogenous organic matter. Various types of organic matter appear to affect the course and intensity of the action differently, and certain of them when used in excess practically inhibit it.

The organism grows very slightly under autotropic conditions and the action on hydrogen is correspondingly very feeble. In conjunction, however, with a second organism which we have isolated, autotropic oxidation of hydrogen takes place; carbon-dioxide forming the necessary carbon source. It is probable that there is here an interesting case of symbiosis, one bacterium providing organic matter for the other.

The experiment detailed in the following table shows the action of this bacterium in 0·02 per cent. sodium asparaginate solution.

TABLE VII.

Showing oxidation of hydrogen by bacterium No. 1 *in mineral nutrient solution to which* 0·02 *per cent. of sodium asparaginate has been added.*

Incubation period 10 days at 32° C.

	Before.	After.	Diff.
CO_2	1·4	2·1	+ 0·7
O	139·0	126·6	−12·4
H	134·0	113·8	−20·2
CH_4	2·3	1·5	− 0·8
N	11·6	12·8	+ 1·2
TOTAL ..	288·3	256·8	−31·5

As a result of this investigation it is evident that in the film there are organisms capable (1) of oxidizing hydrogen in the presence of minute amounts of nitrogenous organic matter, and probably (2) of assimilating carbon-dioxide directly and producing organic matter.

On the function of the surface film.

We have previously drawn attention to the function of the surface film in relation to the aeration of the roots of the rice crop, and also to the effect of green-manuring in increasing the effective aeration. In the absence of green manure the gas production in the soil is comparatively small, and consequently, the green algæ in the film must obtain their necessary carbon dioxide mainly from that dissolved in the irrigation water. When however green manures are used it has been shown that there is a largely increased output of the characteristic soil gases and that concurrent with this, there is an increased activity on the part of the surface film leading to an increased evolution of oxygen.

This relationship between gas production in the soil and oxygen evolution by the surface film we now demonstrate is due to the symbiotic relationships between the oganisms found in the film. The methane mainly, and any hydrogen also which reaches the surface film, is oxidized with the production of carbon-dioxide and water, and the carbon-dioxide is in turn decomposed with the evolution of oxygen. Further, there appear to be organisms present which have the power of assimilating CO_2 and CH_4 under auto-tropic conditions, and the organic matter thus produced is probably utilized by the oganisms responsible for the oxidation processes.

Consequently, in the film there is a community of different organisms which are capable of utilizing to the fullest extent the gases produced in the soil, and one in which the mutual relationships are so elastic as to be eminently capable of utilizing this energy and food-supply under a very great variety of conditions.

The oxygen thus liberated at the surface of the soil is in turn dissolved in the water entering the soil and is utilized for root aeration. In undrained or very badly drained soils this oxygenated water is unable to penetrate into the soil, and the roots as a consequence are restricted to the surface layer and stunted plants and poor crops are produced. In moderately drained soils on the other hand the slow downward current of water is, by contact with the

oxygen-evolving film, more highly aerated than is the case with water in contact with air only, and as a consequence there is a deeper root development resulting in sturdier and more productive plants.[1]

It may possibly be objected that the oxidation processes taking place in the film use up much more oxygen than is recovered from the CO_2 produced, and that consequently there is actually less oxygen available for root aeration than would be otherwise the case. It is undoubtedly a fact that a maximum of only half of the oxygen required to oxidize methane can be liberated by the green algæ, and if the total oxygen supply were limited there would be decreased aeration.

But the oxygen supply to the film is not limited in any sense. These soils are kept covered with a shallow layer of water which is in constant circulation and which being in contact with the air is consequently nearly always saturated with oxygen at the atmospheric partial pressure. This layer of water thus acts as an oxygen carrier to the film and the supply of oxygen to the latter not restricted in the least.

The important aspect of the problem lies, not with the surface layer of water, but with that modicum of water which passes over the surface film and into the soil through the action of drainage. These Swamp Rice Soils are exceedingly heavy in character and the rate at which water drains through them is very slow, and, consequently, the latter, by passing through a film which is actively evolving pure oxygen, becomes more strongly aerated than does the surface water which is merely in contact with the air. The complete picture of the conditions is that of an organization acting as a concentrator of oxygen at the exact point where the most effective aeration of the water which enters the soil can be brought about.

One important result obtained from our experiments on the effect of drainage on the growth of the crop was the fact that

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[1] Harrison and Subramania, *loc. cit.*, p. 101.

drainage exerted a eneficial action only when the rate was low, and that with high rates of drainage deterioration in the cropping occurred.[1] This we attempted to explain mainly on the basis that the strong downward current of water prevented the proper growth of the surface film, but in view of the results of the investigation now presented we feel that this explanation must be modified. That quick drainage does reduce the growth of the film was apparent on inspecting the surface of the soil, but over and above this aspect of the case we believe that the utmost importance attaches to the oxygen concentration in the water entering the soil.

In undrained soils the rate at which water enters the soil will depend solely on the transpiration of the crop. This rate will be extremely slow, but at the same time, the oxygen concentration in the in-going water will be high. The total oxygen, however, which can enter the soil will be small and its action will consequently be limited to the surface layer of soil; and in this layer only will root action be effective. As the rate of natural drainage increases, the volume of water entering the soil increases, and this water will possess the maximum oxygen concentration so long as more oxygen is produced by the film than can be dissolved in the in-going water. Thus, so long as these conditions hold good, the effective aeration of the roots will extend to an increasing depth followed by a corresponding increased cropping, and it is probable that the maximum effect will be reached at the point where the volume of in-going water is just sufficient to dissolve the evolved oxygen. Beyond this point an increasing rate of drainage will result in a lessening oxygen concentration and a decreasing root aeration and cropping; thus agreeing with the cropping results of our pot-culture experiments.

This theory also explains the curious fact that in our undrained and moderately drained pots, the weight of roots produced was practically the same, whereas in the quickly drained pots the weight of roots produced was greatly decreased.[2] After the point of

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[1] Harrison and Subramania, *loc. cit.*, p. 97.
[2] *Ibid*, p. 101.

maximum oxygen concentration is reached, the quick rates of drainage will still permit of deep root development, but the decreased concentration will result in a lessened amount of root production.

We therefore feel justified in putting forward the theory that one of the main factors which affects the growth and yield of the rice crop lies in the oxygen concentration of the water drawn into the soil through the combined action of drainage and transpiration.

Summary.

1. The organized film in contact with the surface of Swamp Rice Soils utilizes the soil gases in such a manner as to bring about an increased oxygen output from the film leading to a correspondingly increased root aeration.

2. The film contains bacteria which possess (1) the power to oxidize methane and hydrogen, and (2) to assimilate directly methane and carbon-dioxide. These changes either directly or indirectly result in the production of CO_2 which is in turn assimilated by the green algæ with the evolution of oxygen.

3. The film may be looked upon as fulfilling the duty of an oxygen concentrator at a point which enables the maximum oxygen concentration to be produced in the water entering the soil.

4. The practice of green manuring by increasing the output of the soil gases brings about an increased activity on the part of the film resulting in an increased oxygen production and root aeration. An important indirect function then of green manuring is to bring about a greater root aeration and so induce greater root development and cropping power.

5. The oxygen concentration of the water entering the soil appears to be one of the main factors which regulates the growth of the crop.

Coimbatore,
June 26th, 1914.

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